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GGE BIPLOT ANALYSIS FOR GENOTYPE X ENVIRONMENT INTERACTION ON YIELD TRAIT OF HIGH FE CONTENT RICE GENOTYPES IN INDONESIAN IRRIGATED ENVIRONMENTS

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ABSTRACT

The prevalence of Iron (Fe) defficiency in Indonesia is around 31 – 63.5 %. High Fe content rice lines had been developed to overcome the problem. This study was aimed to explore the effect of genotype (G) and genotype × environment inter-action (GEI) on yield of 21 high Fe content rice genotypes under 5 irrigated field environments. The research was conducted at DS 2011 in 2 locations and DS 2012 in 3 locations following randomized complete block design with three replications in each location. Combined analysis of variance showed genotype x environment inter-action at 1% probability level, where G and GEI captured totally 88.8% of total variability. There were two Mega-environments constructed, i.e. Mega-E_1 that contained environments of trials in dry season 2011 (E4 and E5) with the winner of G12 (BP9474C-1-1-B) and Mega-E_2 that contained environments of trials in dry season 2012 (E1, E2, and E3) with the winner of G3 (A69-1). E1 (Subang, DS 2012), E2 (Karawang, DS 2012), and E3 (Indramaru, DS 2012) had good discriminativeness and representasiveness for yield trait of high Fe content rice lines. Mean performance and stability of genotypes indicated that G3 (A69-1; average 6.72 t ha^{-1}) was highly stable with high yield.

Keywords: GGE analysis; GEI; stability; paddy

INTRODUCTION

Micronutrient malnutrition is recognized as a massive and rapidly growing public health issue especially among poor people living on an unbalanced diet dominated by a single staple grain such as rice. It was reported that the prevalence of Fe deficiency anemia was estimated to be 55.1% in children under five, 31% in schooling age children, 63.5% in pregnant women, and 35% in manual laborer (Directorate of Public Nutrition, 1993). The problem for women and children is more severe because of their physiological need. Fe deficiency during childhood and adolescence impairs physical growth, mental development and learning capacity.

Breeding staples such as rice with high micronutrient content dubbed as ‘biofortification’ provides a cost effective and sustainable solution to combat malnutrition (Bouis, 2004). Breeding for functional rice had been done in IRRI and ICRR. IRRI had develop high Fe and Zn content rice (Gregorio *et al.,* 2000; Gregorio, 2004). Those lines is predicted to be adapted for Indonesian agro-ecosystem condition and could be then widely planted by farmers.

Yield is a quantitative traits that is strongly affected by environment (Hadi and Sa’diyah, 2004; Rasyad and Anhar, 2007; Widyastuti *et al.,* 2013). GxE study on yield trait of the high Fe and Zn rice lines would give information about the yield and stability, so that it could be selected the best genotypes with high yield and stable accross environment. Some parameters could be used to study the stability, such as regression slope (bi), equivalency (Wi^2), coefficient of determination (Ri^2) and Si^2. Those techniques has been widely used for hybrid rice (Satoto *et al.*, 2013; Widyastuti *et al.*, 2013; Abdullah and Safitri, 2014) and aromatic rices (Akmal *et al.*, 2014) breeding. Nevertheless, visualization of the test would be much helpfull in concluding the results.

GGE biplot analysis is one appropriate tool to evaluate representation of an environment, genotype stability, and the effect of GxE to the performance of a genotype (Yan, 2001; Asfaw *et*

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al., 2009; Jambormias, 2011; Karimizadeh *et al.*, 2013). GGE biplot analysis provides an easy and comprehensive solution to genotype by environment data analysis, which has been a challenge to plant breeders, geneticists, and agronomists. Data does not only address short-term, applied questions but also provides insights on long-term, basic problems (Yan and Tinker, 2006). GGE biplot analysis is a statistical method which used multivariate approach in the analysis. It is better than univariate approach in dissecting GxE components into specific interaction between genotype and environmental components (Flores *et al*., 1998). GGE could explain the source of variation of G (*genotype*) and GE (*genotype* x *environment*) more detail compared to AMMI ana-lysis (Yan *et al.*, 2007). GGE biplot had some graphical visualization function such as visualization of genotypes performance in a specific environment, visualization of relative adaptability of a genotype into various environment, visualization of comparison of two genotypes in different environment, visualization of identifying the best genotypes in every environment condition, visualization of environmental group for a specific genotpe(s), visualization of genotype average performance and stability, and visualization of disccrimination and representation of environment (Yan and Hunt, 2002). GGE biplot is able to show the best genotype with the highest yield in a quadran containing identical locations (Mega-E), genotype average performance and stability, ideal genotype and ideal location to increase yield, and specific location (Jambormias and Riry, 2008; Fashadfar *et al.*, 2013; Fashadfar and Sadegi, 2014).

This research is aimed to know which high Fe content rice lines having wide adapability and the ideal genotypes based on five different locations or year, and to know representation of an environment to select yield based on GGE biplot analysis.

MATERIALS AND METHODS

Twenty one genotypes were tested, consisted of 11 IRRI bred high Fe content rice lines, 5 ICRR bred high Fe content rice lines, 3 IRRI check varieties, and 2 ICRR check varieties (Table 1).

Table 1. High Fe content rice lines for GGE study, Indonesia, DS 2011-2012

No	Genotype	Remarks
1	IR69428-6-1-1-3-3	IRRI lines
2	IR68144-2B-2-2-3-1-166	IRRI lines
3	A69-1	IRRI lines
4	IR83286-22-1-2-1-1	IRRI lines
5	IR84020-84-2-3-2	IRRI lines
6	IR85849-33-1-2-1-2	IRRI lines
7	IR84750-12-1-2-3-1	IRRI lines
8	IR83663-20-3-2-2	IRRI lines
9	IR91143AC-239	IRRI lines
10	IR91152AC-317	IRRI lines
11	IR91152AC-819	IRRI lines
12	BP9474C-1-1-B	ICRR lines
13	BP9458F-19-1-3-B	ICRR lines
14	BP9452F-12-1-B	ICRR lines
15	BP9454F-27-3-2-B	ICRR lines
16	BP9458F-36-8-B	ICRR lines
17	IR64	IRRI check
18	PSBRc82	IRRI check
19	IR78581-12-3-2-2(NSIC222)	IRRI check
20	Ciherang	ICRR check
21	Inpari13	ICRR check

The experiments were conducted in five environments (three locations in dry season of 2012 and two locations in DS 2011), each followed randomized complete block design with three replications (Table 2). The location should had at least a simple irrigation system. Watering was conducted intermittently in every one week and according to the water availability from the irrigation system. The materials were transplanted at 21 days after sowing into 20 cm x 20 cm planting space in 2 m x 5 m plot size. The plant establishment followed the principles of integrated crop management according to local recommendation and condition.

Table 2. Location and year of the trials for high Fe content rice lines in Indonesia

No.	Code	Location	Sowing Time	Season	Remark
1	E1	Pusakanagara, Subang	28 May 2012	Dry Season	Low elevation irrigated field
2	E2	Cilamaya, Karawang	20 June 2012	Dry Season	Low elevation irrigated field
3	E3	Sukra, Indramayu	4 August 2012	Dry Season	Low elevation irrigated field
4	E4	Sukra, Indramayu	June 2011	Dry Season	Low elevation irrigated field
5	E5	Pusakanagara, Subang	19 April 2011	Dry Season	Low elevation irrigated field

Analysis of variance for yield trait was conducted by STAR ver 2.0.1. software, while to determine yield level, stability, environmental representation, the effect of environment (E), genotype (G), and genotype by environment interaction (GEI) by PBTools Ver 1.4.

Combine variance analysis was conducted into yield data of the five locations following the mathematical model as follows (Sumertajaya, 2007):

$$Y_{ij} = \mu + G_i + L_j + GL_{ij} + \varepsilon_{ij}$$

Remarks:

Y_{ij} = yield of genotype i in location j
M = general mean
G_i = effect of genotype-i
L_j = effect of location-j
GL_{ij} = effect of interaction of genotype-i in location-j
ε_{ij} = error

The two biggest effect principle components we used to a biplot by GGE biplot analysis. The basic model for a GGT biplot is:

$$Y_{ij} - \mu - \beta_j = \sum_{i=1}^{k} \lambda_{il}\xi_{ij}\eta_{lj} + \varepsilon_{ij}$$

Where Y_{ij} = the mean yield of genotype i (=1,2,….,n) in environment j (=1,2,…m), μ = the grand mean, β_j = the main effect of environment j, (μ + β_j) being the mean yield of environment, λ_l = the singular value (SV) of lth principal component (PC), the square of which is the sum of squares explained by PCl=(l=1,2,….,k with k≤ min (m,n) and k=2 for a two- dimensional biplot), ξ_{ij}= the eigenvector of genotype i for PCl, η_{lj} = the eigenvector of environment j for PCL, ε_{ij}= the residual associated with genotype I in environment j.

To integrate~~d~~ a biplot that can be used in visual analysis of MET data, the SVs had to be partitioned into the genotype and environment eigenvenctor so that the model (1) could be written in the form of $Y_{ij} - \mu - \beta_j = \sum_{i=1}^{k} g_{il}e_{ij} + \varepsilon_{ij}$ where $g_{il}$and e_{lj}were called PCl score for genotype i and environment j, respectively. In a biplot, genotype i is displayed as a point defined by all g_{il}values, and environment j is displayed as a point defined by all e_{lj}values (l=1 and 2 for a two-dimensional biplot) (Farshadfar *et al.*, 2013).

RESULTS AND DISCUSSION

Combine Variance Analysis

Variance analysis showed that there was significant interaction between genotype and environment on yield trait (Table 3). These data had complied the requirements for biplot analysis. GGE biplot analysis was conducted and viualized to determine the difference of mega-E among the environments, to evaluate stable and wide adapt-able line, and to evaluate the environments if a certain mega-E representing the appropriate envi-ronment to select genotypes based on yield.

Table 3. Combine variance analysis of yield trait of high Fe content rice in 5 sites

Source of variation	Degree of freedom (DF)	Sum of square (SS)	Mean square (MS)	F value	Probability
Location	4	1033.16	258.290**	354.02	0.0001
Replication (location)	10	55.53	5.553**	7.61	0.0001
Genotype	20	157.75	7.888**	10.81	0.0001
Genotype x Location	80	133.83	1.673**	2.29	0.0001
Error	200	145.92	0.730		
c.v %	15.77				

Remarks: **= significant at the confidence level of 1%

Table 4. Analysis of variance of principle components of biplot genotype and location of the trial of high Fe rice lines in five sites, DS 2011 and DS 2012

Principle component	Effect (%)	Accumation (%)	DF	SS	MS	F value	Pr. F
PC1	72.0	72.0	23	277.559	12.067	4.18	0.0000
PC2	16.8	88.8	21	64.955	3.093	1.07	0.3831
PC3	7.9	96.7	19	30.447	1.602	0.56	0.9303
PC4	3.3	100	17	12.601	0.741	0.26	0.9988

GGE biplot analysis identified 4 principle components (PCs) which PC1 had very significant variation (Prob. F < 0.01). PC1 contribute 72 % variation to the total. PC2 contribute 16.8 % to the total variation, with Pr. F value more than 0.005. It means that by using PC1 and PC2, the analysis could explain 88.8 % variation (Table 4).

Mega Environment (Mega-E)

Mega-E is environmental group which has similarity to support performance of some genotypes simultaneously (Crossa *et al.,* 2002). Mega-E is determines by the vertex genotype, i.e. the highest yield genotypes in each quadran developed by GGE analysis visualization (Yan and Hunt, 2002). Position of the vertexs were connected by connecting lines, i.e. a linear line started from the base of biplot that cross perpendicularly each connecting line and separated the biplots into some sectors. Sectors containing environments, i.e. sectors containing dots representing environments, called as Mega-E (Jambormias, 2011).

Based on vertex genotypes and vector lines crosses it was developed five main quadrans (Figure 1). Two out of the five quadrans were mega-E. Mega-E_1 consisted of E4 (Indramayu, DS 2011) and E5 (Subang, DS 2011). Mega-E_2 consisted of E1 (Subang, DS 2012), E2 (Karawang, DS 2012), and E3 (Indramayu, DS 2012). Mega E_1 containing trials during DS 2011, while Mega-E_2 contained environment of DS 2012 trials. Regarding this one, it was predicted that the Mega-E was determined by drought condition of each trial. Trials during 2011 either in Subang and Indramayu were conducted at relatively drought condition, while trials during 2012 were conducted at relataively medium drought condition. Irrigation condition in Subang and Indramayu were relatively limited and Karawang had better irigation supply. On the other hand, rainfall of 2012 trials relatively higher compared to the one of 2011 (Figure 2). Soil type and altitude (below 20 m asl) of the sites were relatively similar. Vertex for Mega-E_1 was G12 (BP9474C-1-1-B) and vertex for Mega-E2 was G3 (A69-1).

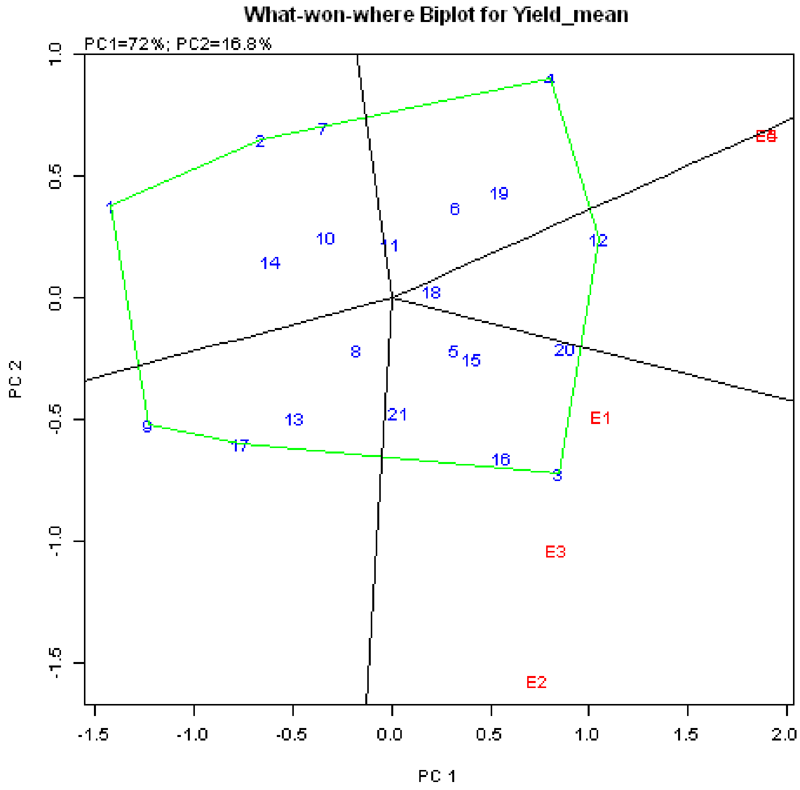


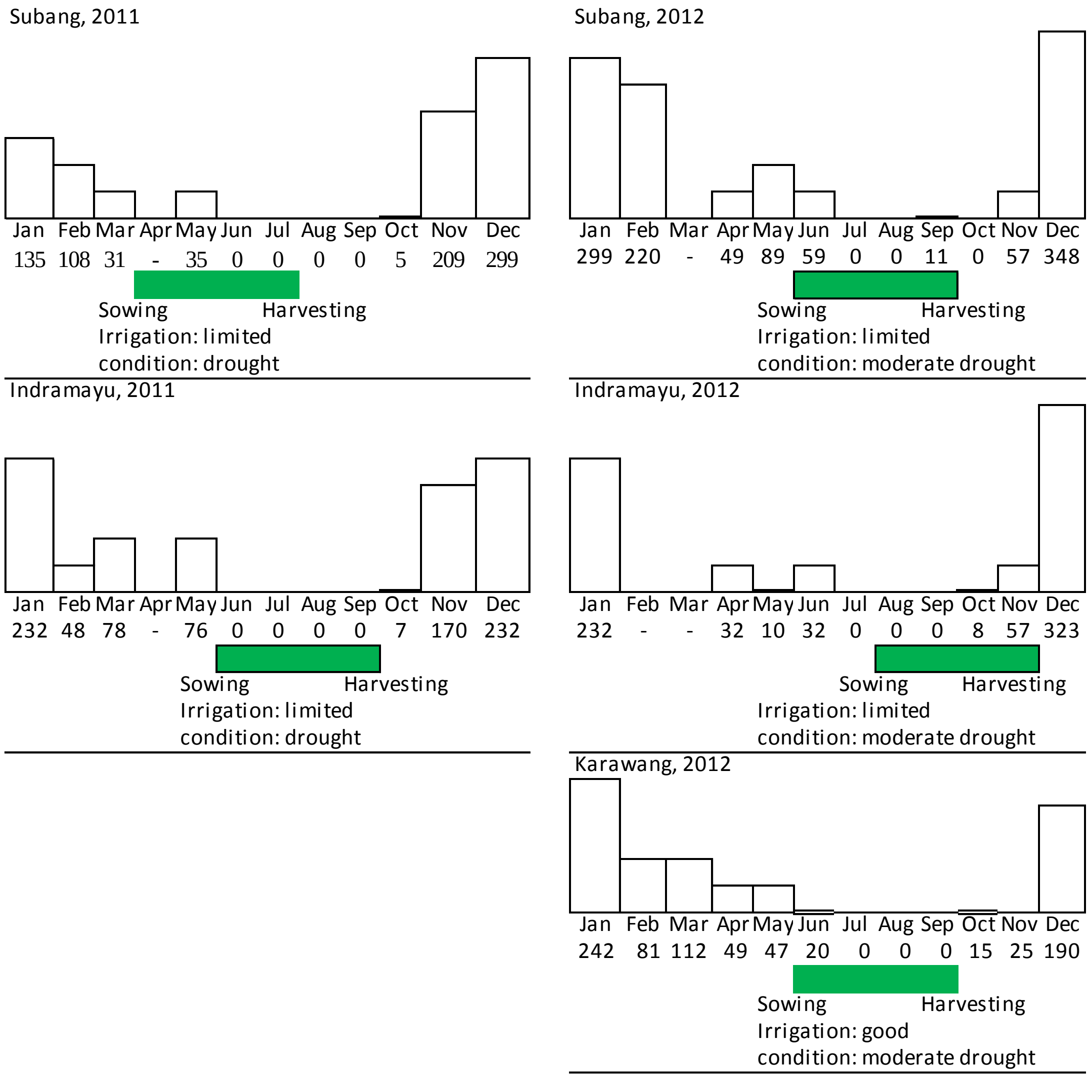
Figure 1. Visualization of the difference of Mega-E on GGE biplot on yield trait of 21 high Fe content rice genotypes in five locations or years, DS 2011 and 2012

Evaluation of Environment

Evaluation of environment is useful to know the appropiateness of an environment that is useful to determine appropiate environment to increase productivity. Evaluation of environment based on GGE biplot analysis (Figure 3) showed that angle of two arrows showed the correlation between two environments. Yan and Tinker (2006) explain that the narrower the angle of two arrows meant the closer the relation between two environments and vice versa. The wider the angle of two arrows meant the more different of the results, because of the bigger effet of GxE of the observed traits.

Vector angle of E5 (Subang, DS 2011) and E2 (Karawang, DS 2012) was 90^0 indicating negative correlation and representation of the sites, thus analysis in both locations would be different. It also indicted the big different of yield of the tested genotypes in both locations. It showed also that GxE interaction was very strong.

It was a different story for E4 (Indramayu DS 2011) and E5 (Subang, DS 2011) which has positive correlation (both environments had narrow angle and both were laid in the same quadran). It indicated that test in E4 and E5 would be relatively gave the same results. It might due to relatively uniform drought condition during DS 2011 in both locations, which were located in north coastal areas of West Java province. They had the same altitute, irrigation availability, and had relatively no long distance of each other (less than 20 km).



Remarks: rainfall criteria 20-100 = low, 101-300 = medium, 301-400 = high, 401-500 = very high (*Source : BMKG 2011 and 2012)*

Figure 2. Rainfall occurence (mm) and drought condition during the trials of high Fe rice lines in five specified sites during DS 2011 and DS 2012

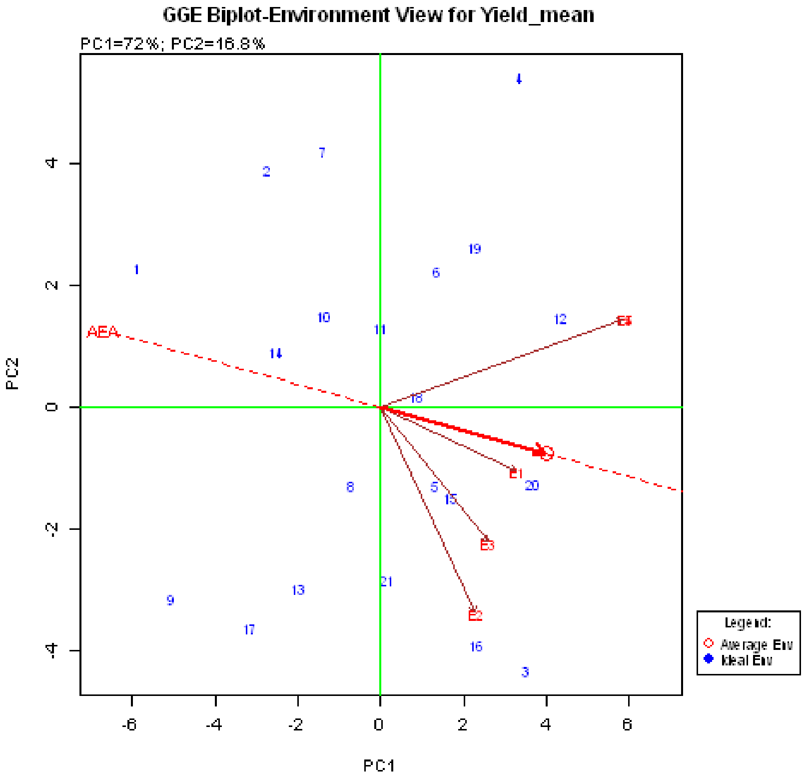


Figure 3. GGE Biplot power to discriminate (*discriminativeness*) and power of representation of an environment (*representasiveness*) of testing of 21 genotypes in 5 environments

Table 5. Standard error of mean and coefficient of variation (CV) of yield trait of 21 high Fe contend rice genotypes in 5 environments (DS 2011 and DS 2012)

Code	Location	Time	St.Error of mean	CV (%)
E1	Subang	DS 2012	0.090167	15.01
E2	Karawang	DS 2012	0.094495	12.60
E3	Indramayu	DS 2012	0.072427	8.94
E4	Indramayu	DS 2011	0.185600	17.38
E5	Subang	DS 2011	0.153479	34.73

GGE biplot analysis showed that there was no location linkage with AEA line (*average environment axis*). Nevertheless, there were 3 environmetal vector lines which were laid in the same quadrant which had the same quadrant with AEA line, i.e. E1, E2, and E3. The narrowest angle fo AEA line was was made by E1. It indicated that E1 (Subang, DS 2012) was the ideal location to discriminate and show the performance of the tested genotypes. Eventhough E2 and E3 had bigger angles to AEA, both locations laid ini the same quadrant and tended to have the same results with E1. Jambormias (2008) reported that locations laid in the quadrant which is the same with AEA vector line had the same tendention of results. It agreed with variance analysis of yield in E1, E2, and E3 which had lower average standard error compared to environment from different quadrant (E4 and E5) (Table 5). Coefficient of variation (CV) of E5 (Subang, DS 2011) was relatively higher compared to other sites. It might indicated that more severe drought condition tend to give higher variation on the observed traits.

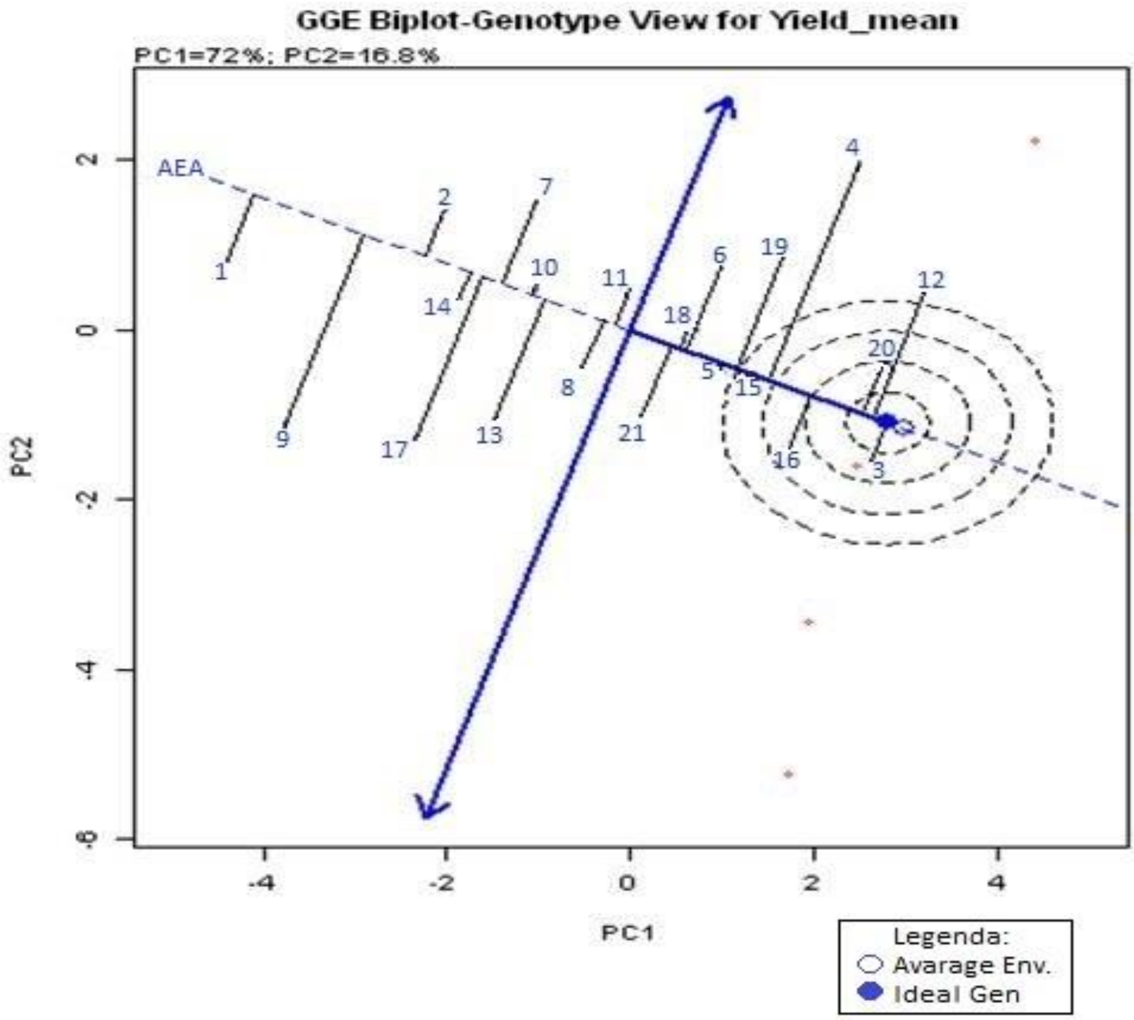


Figure 4. Visualization of GGE biplot showing the stability of genotypes, which linear line showing the axis of environment mean and interupted circle is confidential range

Stability of Genotypes

Visualization of GGE is very useful to evaluate and find the most stable genotypes (Farshadfar *et al*., 2013). Genotypes laid in the concetris area were the more stable in giving the yield compared to the genotypes laid outside, eventhough the environmental effect was very strong.

Based on GGE biplot, G3 (A691) is the ideal genotype (shown by the bold dot in the center of the concentric area). G3 (A691) had highest yield based on the average from all the environment, and it was viusalized by its position in the most right end of AEA line. G3 (A691) had the yield average of 6.72 t/ha (Table 6) with vector deviance relatively not so far from the origin point of the vector, indicating the genotype stability (Figure 4).

There were 6 genotypes laid in the concentric area, i.e. G12 (BP9474C-1-1-B), G20 (Ciherang), G16 (BP9458F-36-8-B), G6 (IR8584 9-33-1-2-1-2), G4 (IR83286-22-1-2-1-1), G15 (BP9454F-27-3-2-B), and G19 (IR78581-12-3-2-2(NSIC222)). G20 (Ciherang) had yield above the average and it is relatively stable. Nevertheless, G3 (A69-1) had relatively higher yield than Ciherang and it was significantly higher than Inpari 13. G3 (A69-1) had highest yield of 6.72 t ha^{-1}, while G20 (Ciherang) had 6.26 t/ha and G21 (Inpari 13) had 5.58 t ha^{-1} (Table 6). The second highest yield genotypes was G12 (BP9474C-1-1-B) with the average yield of 6.40 t/ha. Nevertheless, the deviation of the vector line was very high and the end of the vector line was laid outside the threshold line. It indicated that G12 was not widely adaptable and the yield was not stable accross the environmetns. Based on GGE biplot, G16 (BP 9458F-36-8-B) and G15 (BP9454F-27-3-2-B) had comparable yield and stability with Ciherang. G16 (BP9458F-36-8-B; 6.21 t/ha) had higher yield than G15 (BP9454F-27-3-2-B; 5.83 t/ha), but less stable than G15.

G15 (BP9454F-27-3-2-B) and G5 (IR840 20-84-2-3-2) were the most stable genotypes, indicated by their position which were excactly on the AEA lines having very small vector deviation. It meant that G15 (BP9454F-27-3-2-B) and G5 (IR84020-84-2-3-2) had the widest adaptability and stable yield accross the environment compared to other 19 genotypes. Nevertheless, their yields were not higher than Ciherang. Akmal *et al*., (2014) reported that genotypes with highest yield average was not necesserily be the most stable and vice versa.

Table 6. Yield (t/ha) of 21 high Fe content rice lines in five locations or years, DS 2011 - 2012

No	Genotypes	E1	E2	E3	E4	E5	Average
G1	IR69428-6-1-1-3-3	2.84	5.40	4.19	4.86	2.15	3.89
G2	IR68144-2B-2-2-3-1-166	3.32	5.20	5.16	6.48	1.71	4.38
G3	A69-1	5.92	8.50*	7.57*	8.37	3.22^{+}	6.72*
G4	IR83286-22-1-2-1-1	5.78	5.53	5.95	9.25^{*+}	2.63	5.83
G5	IR84020-84-2-3-2	4.67	7.17	7.09	7.75	2.04	5.74
G6	IR85849-33-1-2-1-2	4.24	6.07	6.85	8.17^{*+}	2.28	5.52
G7	IR84750-12-1-2-3-1	3.93	5.37	5.18	7.09	2.03	4.72
G8	IR83663-20-3-2-2	4.87	7.20	5.67	6.78	2.65	5.43
G9	IR91143AC-239	3.83	6.27	6.03	4.51	1.27	4.38
G10	IR91152AC-317	5.03	5.30	6.15	6.59	2.66	5.15
G11	IR91152AC-819	4.32	5.87	6.76	7.36	2.21	5.31
G12	BP9474C-1-1-B	6.29*	6.53	7.16	9.25^{*+}	2.75	6.40*
G13	BP9458F-19-1-3-B	4.38	6.70	6.86	5.95	1.76	5.13
G14	BP9452F-12-1-B	4.41	4.60	7.16	5.98	2.23	4.88
G15	BP9454F-27-3-2-B	4.88	7.10	7.39*	7.86	1.91	5.83
G16	BP9458F-36-8-B	6.11	7.27	8.17^{+*}	7.70	1.78	6.21*
G17	IR64	4.14	6.47	7.02	5.34	2.85	4.83
G18	PSBRc82	4.37	6.17	7.53*	7.66	2.94	5.71
G19	IR78581-12-3-2-2(NSIC222)	4.62	6.03	6.99	8.60	2.14	5.84
G20	Ciherang	5.37	7.97	6.95	8.86	1.85	6.26
G21	Inpari13	4.99	7.80	6.20	7.03	2.15	5.58
	Average	4.68	6.40	6.57	7.2	2.20	5.41
	LSD 5%	1.16	1.33	0.97	2.07	1.26	0.61

Remarks: E1 = Subang, DS 2012, E2 = Karawang, DS 2012, E3 = Indramayu, DS 2012, E4 = Indramayu, DS 2011, E5 = Subang, DS 2011. LSD = Least Significant difference, * = significantly different from Inpari 13, $^{+}$ = significantly different from Ciherang

CONCLUSIONS

G3 (A69-1, 6.72 t/ha) had highest yield acrross the environmets with the stability comparable to G20 (Ciherang; 6.26 t/ha) and more stable compared to G21 (Inpari 13; 5.58 t/ha). A69-1 is thus prospective to be further tested.

G15 (BP9454F-27-3-2-B; 5.83 t/ha) and G5 (IR84020-84-2-3-2; 5.74 t/ha) had high stability due to no deviation between their vector line to the base of AEA line. Nevertheless, the yield were not the highest.

E1 (Subang, DS 2012), E2 (Karawang, DS 2012), and E3 (Indramaru, DS 2012) had good discriminativeness and representasiveness for yield trait of high Fe content rice lines, due to narrow angle of environmental vector arrow to the line of AEA (average environment axis) and locted in the same quadrant with AEA line.

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